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Study on Ultrasonic-assisted Extraction of Polysaccharide of *Atractylis Macrocephala* Koidz of Experiment¹

BI Yong-guang , YANG Ding-long, Huang XIAO-jun, LI Yu-min, HUANG Min-xia

College of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, Guangdong, China

Abstract

Objective: To optimize the polysaccharides *Atractylodes* Ultrasonic extraction process. **Methods:** The impact of orthogonal design of the ultrasonic extraction of polysaccharides *Atractylodes* main factors, and determine the best extraction. And compared with conventional extraction method. **Results:** The results show that the optimal conditions: ultrasound temperature is 55 °C, ultrasonic power 400W, solid to liquid ratio of 1:30, ultrasonic time of 10min, the optimum conditions, the extraction rate of polysaccharides *Atractylodes* is 15.13%, extraction impregnation 180min Rate of 3.84% the conventional mixing of the extraction rate 9.43% 150min. Conclusion: Compared with the traditional extraction methods, the ultrasonic extraction with time-saving, energy saving, high efficiency and so on..

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Key words: Polysaccharide of *Atractylis macrocephala* koidz ;Ultrasonic; Extraction; Orthogonal experiment

1.Introduction

Ultrasonic extraction technique was developed by acoustic and chemical cross-cutting and penetration of traditional Chinese medicine evolved, used in pharmaceutical, chemical, oil, food and other fields, especially in the extraction of medicinal ingredients in demonstrating its strong vitality. Ultrasound is a body of water and other media, and has good penetration, and the shock wave propagates as a mechanical energy. Ultrasonic extraction, also known as ultrasonic extraction, ultrasonic-assisted extraction. The principle of ultrasonic extraction include mechanical effects, thermal effects and cavitation, is the use of ultrasonic radiation generated by intense cavitation, mechanical vibration, disturbance effects, high acceleration, emulsifying, spreading, crushing and mixing a variety of roles

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increase the frequency of material and speed of molecular motion, solvent penetration, thus speeding up the target component into the solvent. Ultrasonic extraction can greatly improve the extraction efficiency, saving the solvent, to avoid the effects of temperature on the extraction composition. Chinese herbal ingredients is complex and impurities, interference, so the sample preparation, extraction of technically demanding components. Preparation of technical analysis behind the development of traditional Chinese medicine has been restricting the "bottleneck" one. Chinese medicine samples before processing heavy, cumbersome, time-consuming, ultrasonic extraction technology can streamline operations, reduce processing time, increase analysis speed^[1-2].

Atractylodes is a traditional Chinese herbal medicine, it has anti-ulcer, enhance immune function, enhance hematopoietic function, diuretic, inhibit uterine contraction, anti-oxidation, anti-aging, lowering blood sugar, anti-tumor, and many other pharmacological effects^[3]. Atractylodes polysaccharides, total sugar Atractylodes quite prominent, which is an important biological polysaccharide polymers, is the major antioxidant activity of plant components. Can be seen looking for a high efficiency of extraction of polysaccharides Atractylodes methods in terms of economic and medical health are of great benefit. This study focuses on the ultrasonic extraction technology in the extraction of polysaccharides Atractylodes extract is superior to other methods, and ultrasonic extraction by orthogonal test to determine the optimal conditions for process optimization.

2. Materials

2.1. Experimental materials

Atractylodes purchased from Guangzhou Qingping market, crushed dried after use.

2.2 Reagents

Ethanol, 95% ethanol, acetone, phenol, sulfuric acid, the above reagents were of analytical grade.

2.3 Apparatus and equipment

HH - 6 heated water bath (Jiangsu Jintan Honghua Instrument Factory); RE-52CS rotary evaporator (British Yu Gongyi City to China Instrument Factory); to CHINABRAND circulating water pump (British Yu Gongyi City, Henan Province to China Instrument Factory); UV1101 UV / visible spectrophotometer (Shanghai Tian Mei Scientific Instrument Co., Ltd.); AY120 electronic analytical balance (Shimadzu, Japan); KH-400KDB high-power digital ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd. Wo Chong) ; swing-speed Chinese medicine grinder (Dade Chinese Machinery Co., Ltd.).

3. Experimental method

3.1 Preparation of standard solutions of glucose

Precision Weigh glucose reference substance 25.0mg, home 50.0mL volumetric flask, add distilled water and diluted to the mark. Shake, that was glucose control solution (0.500mg/1mL).

3.2 Preparation of standard curve

Precision Weigh glucose reference substance 25.0mg, dissolved in distilled water and set the volume to 50.0mL, to a concentration of 0.500mg/mL. Were taken 0,1.0,2.0,3.0,4.0,5.0,6.0 mL glucose

solution diluted with distilled water to volume 25.0mL flask with a pipette from each 1.0mL in a test tube, under the conditions of the ice water bath, slowly of 5% phenol solution, 1.0mL, shake, quickly adding concentrated sulfuric acid solution 5.0mL, shake 5min, boiling water bath for 10min, and then set the cold bath to cool 20min, while the phenol - sulfuric acid solution as the blank control, at 490 nm absorbance value is measured. To absorb the value of A for the vertical axis, the concentration C as the abscissa the standard curve, the regression equation $y = 40.686x - 0.0038$, $R = 0.9944$ ($n = 6$). The results showed that glucose levels in 0.0000020 ~ 0.000012g/mL absorption within the range of values of a good linear relationship.

Atractylodes

3.3 Preparation and determination of polysaccharides

Weigh 2.000g → Atractylodes powder into the round bottom flask, add appropriate amount of distilled water and mix → set by various conditions (time, power, liquid-solid ratio, temperature) within the ultrasonic cleaner to extract → vacuum filtration (50ml distilled water) → → extracted filtrate was concentrated to 1 / 5 → plus the amount dissolved in distilled water, and placing 25mL flask, diluted to the scale, precision drawing a certain amount of sample solution (by dilution to determine the uptake) set 100mL flask, add water to the mark, shake. Precision drawing solution, 1.0mL, 1.0mL of distilled water for precision drawing another blank control, placed in a test tube, add 5% phenol solution, 1.0mL, 5.0mL concentrated sulfuric acid, shaken, heated in boiling water bath for 10 min, cooled, measured at 490nm wavelength absorbance values, the measured yield and polysaccharide content.

3.4 polysaccharides Atractylodes extract the calculation of the rate EY

$$EY = \frac{x_t(mg)}{x_0(g)} \times 100\%$$

Where x_0 represents the amount of extract before Atractylodes, x_t stands for the amount of polysaccharides extraction

4. Results and discussion

4.1 Single-factor experiment and the results

- Ultrasonic inspection time on the extraction rate

Weigh 2.000g Atractylodes powder 6 copies, in the solid-liquid ratio of 1:20, ultrasonic power 280W, under the conditions of temperature 30 °C, extraction time 10,20,30,40,50,60 min study on the effect of polysaccharide extracted .

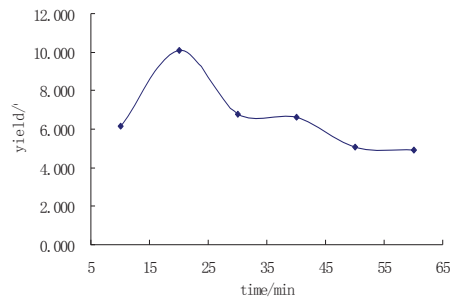


Figure 1 ultrasonic time Atractylodes extraction yield of polysaccharides

Figure 1 shows that, with the ultrasonic treatment time, the extraction of polysaccharides Atractylodes first increase and then drop to flat. Short time (less than 20 min) ultrasonic treatment at a lower rate of extraction; extend the processing time to 20 min, the extraction rate increased rapidly and reached its peak (10.081%). Additional time, the extraction rate decreased rapidly. The reason is the beginning of the extraction process, a large solid-liquid density difference, diffusion driving force, with the extension of time to extract the water temperature will rise faster to promote hydrolysis of polysaccharides, will increase the impurity content, lower polysaccharide content.

- Examine the ultrasonic power on the extraction rate of

Weigh 2.000g Atractylodes powder five, 20 in the solid-liquid ratio 1:1, extraction time 15 min, extraction temperature 30 °C under the conditions of ultrasonic power 200,240,320,360,400 W study on the effect of polysaccharide extracted

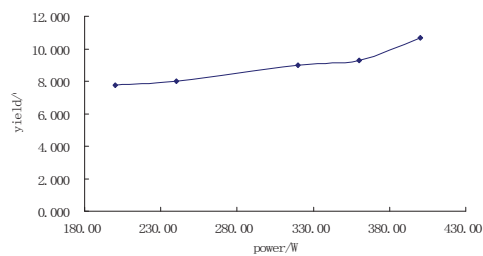


Figure 2 ultrasonic power Atractylodes extraction yield of polysaccharides

Figure 2 shows that in the ultrasonic power 200W-400W range, the extraction rate of polysaccharides Atractylodes increased with ultrasonic power and increase when the ultrasonic power is 400W, the rate of 10.665% polysaccharides. Discovered through testing, ultrasonic power the greater the extraction rate of polysaccharides Atractylodes higher, mainly due to the greater power of ultrasound, cavitation and mechanical effects more intense, the greater the diffusion rate, the faster the polysaccharide exudation. But with the ultrasonic power increases, the extraction rate of polysaccharides Atractylodes increase gradually decrease was mainly Atractylodes polysaccharide content decreased, more and more insoluble out.

- Examine the extraction temperature on extraction yield

Weigh 2.000g Atractylodes powder 6 copies, in the solid-liquid ratio of 1 : 20, extraction time, 15min, 280W power ultrasonic extraction conditions, the temperature of 30,35,40,45,50,55 °C study the extraction of polysaccharides impact.

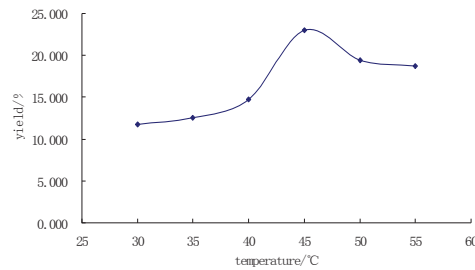


Figure 3 Temperature Atractylodes extract the extraction yield of polysaccharides

Figure 3 shows, as the temperature rises, the extraction rate of polysaccharides Atractylodes gradually increased, extraction temperature 45 °C, the highest extraction rate (23.051%), then the extraction rate decreased slowly and leveling. Thus, with increasing temperature, ultrasonic treatment, the extraction of polysaccharides Atractylodes first increase, then decreased, probably due to the treatment temperature is too high, so that part of the polysaccharide degradation, resulting polysaccharides Atractylodes extract rate.

- Examine the solid-liquid ratio on the extraction rate of

Weigh 2.000g five Atractylodes powder, the extraction time, 15min, extraction temperature 30 °C, 280W power ultrasonic extraction conditions, the liquid feed ratio 1:10,1:15,1:20,1:25,1 study : 30 effect of polysaccharide extracted.

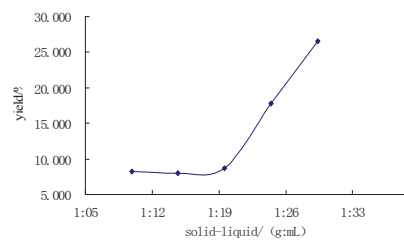


Figure 4 solid-liquid extraction of rate of polysaccharides

From Figure 4, the beginning of the increase of the amount of solvent to improve the extraction rate of polysaccharides little effect, when the solid-liquid ratio of 1:20, with the solid-liquid ratio increases, the extraction rate of polysaccharides Atractylodes liquid increases, which may be due to: a small amount of solvent exudation of polysaccharides will soon be saturated, the diffusion driving force is small, not conducive to the spread, making the extraction of ultrasound have hindered, when the solvent is large enough, the ultrasonic extraction basic non-blocking effects, the extraction rate increased significantly. Taking into account the economic benefits of the solvent and subsequent use of energy technology issues, in the appropriate range of small solid-liquid more appropriate choice.

4.2 Orthogonal experimental results and analysis

Orthogonal to each experiment the amount of Atractylodes is 2.000g. To ultrasonic extraction time, ultrasonic power, solid-liquid ratio and extraction temperature factors, each set three levels of design (see table 1), polysaccharides Atractylodes extract ultrasonic inspection process, the results shown in table 2, table 3.

Table 1 Ultrasonic extraction of polysaccharides *Atractylodes* orthogonal head design

	Factor			
	Time min (A)	Power W (B)	Temperature °C (C)	Solid-liquid ratio g/mL (D)
1	10	200	35	1 : 20
2	20	320	45	1 : 25
3	30	400	55	1 : 30

Table2 Ultrasonic extraction of polysaccharides *Atractylodes* orthogonal experimental results

Number	A/ min	B/W	C/°C	D/ g:mL	yield/%
1	1	1	1	1	9.52
2	1	2	2	2	13.40
3	1	3	3	3	16.66
4		1	3	2	13.39
5	2	2	1	3	13.51
6	2	3	2	1	6.72
7	3	1	2	3	11.91
8	3	2	3	1	5.77
9	3	3	1	2	11.60
K1	13.19	11.55	11.61	7.34	
K2	11.21	10.68	10.90	12.80	
K3	9.76	11.94	11.66	14.03	
k1	4.397	3.849	3.869	2.446	
k2	3.736	3.559	3.631	4.266	
k3	3.255	3.979	3.887	4.676	
R	3.427	1.260	0.768	6.690	

Table 3 Ultrasonic extraction of polysaccharides *Atractylodes* orthogonal analysis of variance table

factor	square	F	threshold	Significance
A	17.76	16.12	19.00	
B	2.49	2.26	19.00	
C	1.10	1.00	19.00	
D	76.11	69.06	19.00	*
error	1.10			

As can be seen from table 2, 3, affecting ultrasonic extraction of polysaccharides *Atractylodes* four factors (extraction temperature, ultrasonic power, solid-liquid ratio, reaction time), the solid-liquid ratio of greatest impact, the impact of ultrasonic temperature minimum, the impact of polysaccharide The order of the extraction rate has to: D> A> B> C, the extraction of solid-liquid ratio> extraction time> Ultrasonic Power> temperature. Comprehensive consideration of economic factors, the factors obtained for the optimization of process parameters: extraction time, 10min, extracted power 400W, extraction

temperature 55 °C, solid-liquid ratio 1:1 30. By the variance analysis shows that the ultrasonic polysaccharides Atractylodes extract orthogonal factors in the choice and level range, the ratio reached a significant level, while the extraction temperature, ultrasonic power, reaction time was not significant.

4.3 Experimental verification

Weigh Atractylodes powder 2.000g, the extraction time 10min, extracted power 400W, extraction temperature 55 °C, solid-liquid ratio 1:30. Repeated three times under the experimental conditions were measured polysaccharides Atractylodes extract was 14.53%, 16.00% and 14.86%, the average extraction of 15.13%.

4.4 Ultrasonic and conventional extraction method of comparative

Ultrasonic extraction by 3.2 the best extraction process, with conventional mixing method and impregnation method for comparison polysaccharides Atractylodes extract. Conventional stirring extraction: precision that Atractylodes 2.000g, mixing time 150 min, room temperature extraction, solid-liquid ratio 1:1 extract 20; impregnation extraction: precision that Atractylodes 2.000g, dipping time of 180 min, extraction temperature at room temperature, solid-liquid ratio 1:20.

Table 4 Comparison of three extraction methods

	C	B	D	A	Yield/%
Impregnation	30	/	1 : 30	180	3.84
Mixing	30	/	1 : 30	150	9.43
Ultrasonic	55	400	1 : 30	10	15.13

From Table 4, experimental results, the ultrasonic extraction and impregnation extraction, stirring to extract the maximum difference between the performance of the extraction time and extraction rate of both. Ultrasonic extraction of polysaccharides Atractylodes extract rate is 10 minutes to extract 3 hours dipping more than 4 times 2 is 150 minutes of stirring times. It showed ultrasonic extraction compared to conventional extraction methods have a greater advantage.

5. Discussion

Experimental results show. Polysaccharides Atractylodes affecting ultrasonic extraction of the four factors (temperature, ultrasonic power, extraction of solid-liquid ratio, extraction time), the solid-liquid ratio of greatest impact, the impact of ultrasonic temperature minimum, the impact of the extraction rate of polysaccharides has the size of the order is: extract solid-liquid ratio> extraction time> Ultrasonic Power> temperature. Comprehensive consideration of economic factors, the factors obtained for the optimization of process parameters: extraction time, 10min, ultrasonic power 400W, extraction temperature 55°C, solid-liquid ratio 1:30. Polysaccharides in the best conditions to extract rate is 15.13%. Compared with the traditional methods of water extraction, ultrasonic extraction with time-saving, energy saving, high efficiency and other advantages, this process simple and feasible, is a polysaccharide extract of Atractylodes effective way.

Acknowledgments

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